Reference: *Biol. Bull.* 180: 466–474. (June, 1991)

On the Nature of Paddle Cilia and Discocilia

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Abstract. Cilia with paddle-shaped or disc-shaped tips enclosing a curved end of the axoneme (paddle cilia or discocilia) have been described in a variety of marine invertebrates. Although numerous studies, in which fixed specimens were used, claimed that paddle cilia and discocilia are genuine structures of unknown function, several studies, in which fresh living material was used, reported that modified cilia are artifacts. We have re-investigated a recent SEM report that paddle cilia are genuine organelles in veliger larvae of marine bivalves (Campos and Mann, 1988). Using high-speed video and electronic flash DIC microscopy, we find no paddle cilia in living larvae of *Spisula solidissima* and *Lyrodus pedicellatus*. Hypotonic seawater, however, induces formation of paddle cilia and vesiculations of the ciliary membrane in these veligers, as does the hypotonic SEM fixative used by Campos and Mann (1988). Fixatives that are isosmotic with seawater, on the other hand, do not induce paddle cilia. We conclude that paddle cilia are artifacts, and we propose a unifying mechanism to explain their production in various animals under different conditions.

Introduction

Cilia with a distal expansion of the ciliary membrane enclosing a looped end of the axoneme (paddle cilia or discocilia) have been described in a variety of marine invertebrates (Tamarin *et al.*, 1974; Oldfield, 1975; Bergquist *et al.*, 1977; Dilly, 1977a, b; Ehlers and Ehlers, 1978; Heimler, 1978; Storch and Alberti, 1978; Arnold and Williams-Arnold, 1980; Bone *et al.*, 1982; Matera and Davis, 1982; Pfannenstiel, 1982; Nielsen, 1987; Campos and Mann, 1988; Durfot *et al.*, 1990). In spite of older cytological evidence to the contrary (Hartmann, 1953; Lewin and Meinhart, 1953; Preer and Preer, 1959; Child, 1961; Pitelka and Child, 1964), many investigators believe that paddle cilia are genuine organelles. Various functions have been proposed for paddle cilia, including serving as micro-spatulae for application of adhesive material or secretions to the substrate (Tamarin *et al.*, 1974; Dilly, 1977b), increasing the efficiency of the power stroke and the effectiveness of water and feeding currents (Bergquist *et al.*, 1977; Dilly, 1977a; Arnold and Williams-Arnold, 1980), increasing membrane surface area for trapping food particles (Dilly, 1977a), acting as chemoreceptors (Matera and Davis, 1982; Campos and Mann, 1988), and transporting unknown materials along the cilium from base to tip (Dilly, 1977a, b).

However, the few studies that have carefully compared fresh living material to chemically fixed or quick-frozen material have concluded that paddle cilia and discocilia are artifacts caused by osmotic stress, non-physiological conditions, or fixatives and fixation additives (Ehlers and Ehlers, 1978; Bone *et al.*, 1982; Pfannenstiel, 1982; Nielsen, 1987).

To examine the status of paddle cilia anew, we have re-investigated a recent SEM report that paddle cilia are genuine structures in veliger larvae of marine bivalves (Campos and Mann, 1988). We used high-speed video and electronic flash DIC microscopy of living larvae of *Spisula solidissima* and *Lyrodus pedicellatus* in normal seawater and in hypotonic seawater, together with light microscopy and SEM of larvae fixed in solutions of different osmolarities and composition.

We find that paddle cilia are indeed artifacts, and propose a new mechanism to account for their formation in various animals. A preliminary account of this work has appeared (Short and Tamm, 1989).

Materials and Methods

Organisms

Spisula solidissima adults were obtained from Marine Resources at the Marine Biological Laboratory (MBL)

Received 7 February 1991; accepted 22 March 1991.

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and maintained in cold running seawater (15°C) at the Environmental Studies Laboratory (ESL) of the Woods Hole Oceanographic Institution (WHOI); *Lyrodus pedicellatus* larvae were obtained from adults maintained at ESL. *Spisula* adults were spawned by dissection of gonads or by thermal stimulation at 22°C, and the larvae reared following methods of Gallager and Mann (1986). Larvae were fed monocultures of *Isochrysis galbana* at a concentration of 10,000 cells per ml.

Light microscopy and video recording

Living veliger larvae of *Spisula solidissima* and *Lyrodus pedicellatus* were observed in slide wells of normal seawater under Zeiss DIC and phase contrast optics with a Dage 67 video camera modified for high field rates (120, 180, 240 Hz) and synchronized with a strobex flash (Chadwick-Helmuth). Images were recorded with a GYYR model 2051 video recorder allowing still-field playback and analysis. Films (35 mm; Kodak Tech Pan 2415) of larvae were taken with Zeiss DIC and phase contrast optics using an Olympus OM-2N camera and an Olympus T-32 electronic flash tube positioned in the illumination path.

Fixation and scanning electron microscopy

Umbo stage larvae of *Spisula* and *Lyrodus* were rinsed in 0.45 μm filtered seawater and fixed in three ways. *Method 1* (Campos and Mann, 1988): larvae were siphoned from the culture container and retained on a 50 μm nylon mesh screen, then transferred to filtered seawater and relaxed in 8% (w/v) $MgCl_2$. Larvae were concentrated by centrifugation and fixed in 2.5% glutaraldehyde, 0.1 *M* Na cacodylate, pH 7.2 (total osmolarity of 409 mOsmols as determined by Wescor 5100C vapor pressure osmometer) at 4°C for 2 h. Larvae were rinsed 3 times in 0.1 *M* Na cacodylate, 0.25 *M* NaCl, pH 7.2, for 30 min each, and post-fixed in 1% OsO_4, 0.19 *M* NaCl, 0.1 *M* Na cacodylate for 1 h. Larvae were rinsed in 0.1 *M* Na cacodylate, 0.15 *M* NaCl and stored overnight at 4°C. *Method 2:* the same glutaraldehyde solution as above was used, but with 0.29 *M* NaCl added to make it isosmotic with MBL seawater (920 mOsmols). *Method 3:* concentrated larvae were relaxed in 6.82% $MgCl_2$ and fixed in unbuffered 2.5% glutaraldehyde, 0.13 *M* NaCl, 50% seawater (isosmotic; 920 mOsmols) at 4°C for 30 min.

For light microscopy, larvae were observed on slides after glutaraldehyde fixation. For SEM, fixed larvae were dehydrated through a graded ethanol series, critical point dried (Samdri-78A), sputtered with gold palladium (Samsputter-2a), and examined with a JSM-840 SEM. Photographs were taken on Polaroid positive-negative film.

Results

Light microscopy of living and fixed larvae

The velum of *Spisula solidissima* and *Lyrodus pedicellatus* consists of four ciliary bands: inner and outer pretrochal bands, an adoral band, and a metatrochal band. The pretrochal bands are responsible for obtaining food and for locomotion, and the adoral and metatrochal bands convey food particles to the mouth. The pretrochal bands consist of compound cilia; the adoral and metatrochal bands contain simple cilia. Figures 1A and 1B are flash photographs of living umbo stage larvae of *Spisula* (two weeks old) and *Lyrodus* (two days old), respectively, in normal seawater. No paddle cilia or vesiculated ciliary membranes are evident in any of the ciliary bands of either larva. High-speed flash-synchronized video microscopy of swimming *Spisula* and *Lyrodus* larvae also failed to show modifications of ciliary structure.

The hypotonic glutaraldehyde fixative of Campos and Mann (1988) (Method 1), with or without OsO_4 postfixation, induced swelling of the tips of pretrochal cilia of *Spisula* larvae (Fig. 1C) and vesiculation along pretrochal ciliary membranes of *Lyrodus* larvae (Fig. 1D). The terminal swellings of *Spisula* cilia and vesiculations along the shafts of *Lyrodus* cilia were about 2 μm in diameter. Because these modifications of ciliary structure were observed directly in fixed larvae by light microscopy, they are not induced by subsequent procedures used for SEM and TEM.

Addition of NaCl to make this fixative isosmotic with MBL seawater (Method 2) resulted in no paddle cilia in *Spisula* larvae, nor vesiculation of ciliary membranes in *Lyrodus* larvae. Instead, the cilia appeared uniformly smooth and cylindrical. Similarly, an unbuffered isosmotic glutaraldehyde fixative containing 50% seawater (Method 3) did not induce paddle cilia or vesiculations in either species (Fig. 1E, F).

Treatment of living, 2-week-old larvae of *Spisula* with 45% seawater (420 mOsmols) caused swelling of the distal tips of the pretrochal cilia within 2 min. These paddle cilia resembled those induced by hypotonic fixatives (Fig. 1C). Treatment of *Lyrodus* veligers with 45% seawater resulted in vesiculation of the membrane along the entire shaft of the pretrochal cilia within 10 min. Again, these vesiculated cilia resembled those induced by hypotonic fixatives (Fig. 1D). The majority of the modified cilia in both species remained attached to the velum. However, treatment with 45% seawater for longer times caused detachment and loss of cilia. Upon transfer of larvae to 100% seawater, many cilia of both species regained their normal appearance within 5–10 min, indicating that tip swelling or vesiculation is a reversible osmotic phenomenon.

In a subsequent experiment using 2-day-old *Spisula* veligers, we found that 45% seawater was ineffective in producing paddle cilia, but that 15–20% seawater was re-


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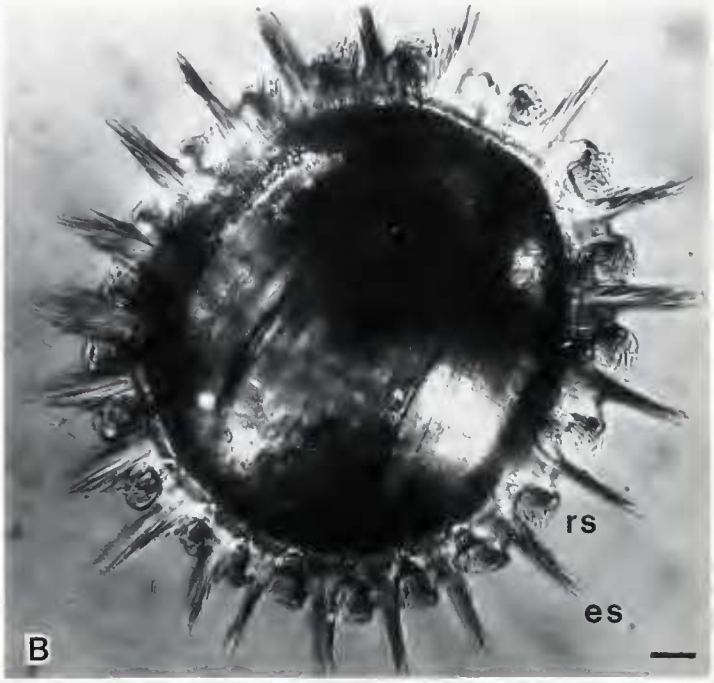
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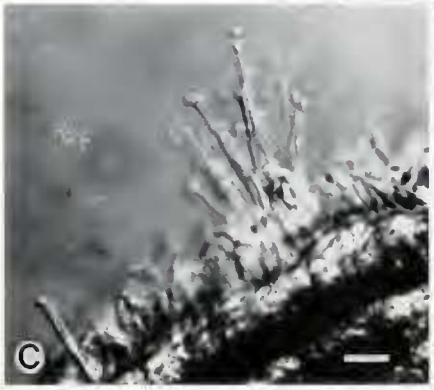
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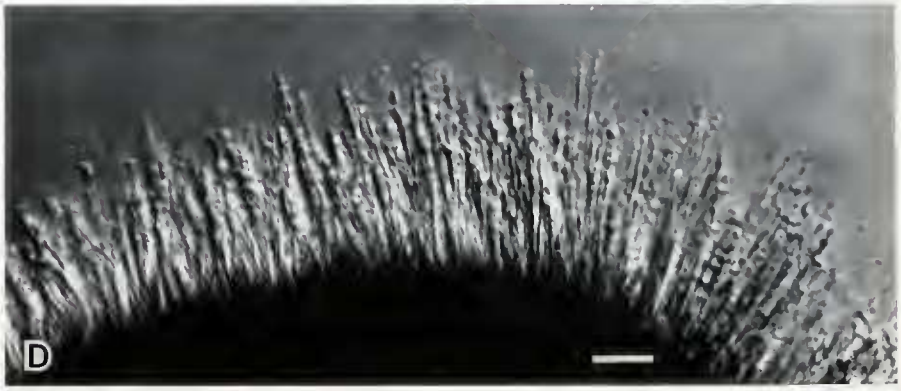
D


E


F

quired to induce swelling of the ciliary tips in these younger larvae. The paddle cilia were immotile or only weakly beating and were easily detached, resulting in poor swimming ability of the larvae. Upon transfer to 100% seawater, many of the larvae resumed swimming. DIC microscopy of these larva showed that some velar cilia regained a normal appearance, but that others had detached and were missing.

Scanning electron microscopy of larvae

Larvae of *Spisula* and *Lyrodus* treated with the isosmotic fixative containing 50% seawater (Method 3) and processed for SEM, showed uniformly cylindrical velar cilia without terminal swellings or vesiculations (Figs. 2A, C, 3A, C). However, swollen cilia were present in both species when fixed by the hypotonic fixative of Campos and Mann (1988) (Method 1) (Figs. 2B, D, 3B, D). In contrast to light microscopic images of Method 1-fixed *Lyrodus* larvae (Fig. 1D), those processed for SEM showed terminal paddles on pretrochal cilia rather than vesiculation along the ciliary length (Fig. 3B, D). The modified cilia induced in *Spisula* and *Lyrodus* are similar to those observed by Campos and Mann (1988). The distal swellings measure 1–1.15 μm in diameter in both species, and often result in fraying of the compound organelles into individual cilia. The paddle cilia observed in *Spisula* are not restricted to the pretrochal ciliary bands; metatrochal cilia also exhibit terminal swellings in response to the hypotonic fixative of Campos and Mann (1988) (Fig. 2B). However, the metatrochal ciliary blebs measure about 1.0 μm in diameter and are located about 1 μm proximal to the ciliary tips. The adoral cilia, in contrast, do not exhibit dilations at the tips (Fig. 2B).

Discussion

We have reinvestigated the report by Campos and Mann (1988) that paddle cilia and discocilia are genuine structures in the velum of molluscan bivalve larvae. Campos and Mann (1988) did not examine living larvae, but used a hypotonic fixative (409 mOsmols; Method 1) to prepare larvae of *Spisula solidissima* and *Mullina lateralis* for SEM.

We imaged beating velar cilia in larvae of *Spisula solidissima* and *Lyrodus pedicellatus* by electronic flash and high-speed video light microscopy. No paddle cilia or discocilia were observed in normal seawater. Other high-speed video microscopic studies also have not found modified cilia in living larvae of *Spisula, Lyrodus,* and *Mercenaria* (Gallager, 1988; pers. comm.).

We could reversibly induce swelling of the ciliary membrane by treatment of living larvae with hypotonic (15–45%) seawaters. In addition, paddle cilia were observed using the hypotonic glutaraldehyde fixative of Campos and Mann (1988) (Method 1), but not in fixatives made isosmotic with seawater (Methods 2 and 3). We therefore conclude that paddle cilia and discocilia in *Spisula* and *Lyrodus* are not genuine structures, but are artifacts.

Of the numerous reports of paddle cilia and discocilia in various animals (Mecklenburg *et al.*, 1974; Tamarin *et al.*, 1974; Oldfield, 1975; Bergquist *et al.*, 1977; Dilly, 1977a, b; Ehlers and Ehlers, 1978; Heimler, 1978; Storch and Alberti, 1978; Arnold and Williams-Arnold, 1980; Bone *et al.*, 1982; Matera and Davis, 1982; Pfannenstiel, 1982; Nielsen, 1987; Campos and Mann, 1988; Durfot *et al.*, 1990), only a handful of investigators concluded that modified cilia are artifacts (Mecklenburg *et al.*, 1974; Ehlers and Ehlers, 1978; Pfannenstiel, 1982; Bone *et al.*, 1982; Nielsen, 1987). These investigators, in contrast to the others, did not rely mainly on fixed material, but used fresh living specimens and compared the effects of stress and various TEM and SEM preparative procedures on ciliary structure.

For example, Ehlers and Ehlers (1978) found that living, untreated marine Turbellaria do not possess paddle cilia or discocilia, but that these structures could be induced by the addition of certain fixative buffers and chemicals to the seawater. Osmolality also influenced the extent of paddle cilia formation (Ehlers and Ehlers, 1978).

Similarly, Pfannenstiel (1982) did not observe modified cilia in living polychaetes, but could produce paddle cilia or discocilia by glutaraldehyde and osmium fixatives, or by $MgCl_2$ solutions of different osmolarities. When $MgCl_2$-treated specimens were returned to seawater, the modified cilia regained their normal cylindrical appearance, "revealing that they are transient structures" (Pfannenstiel, 1982).

In addition, Bone *et al.* (1982) found that the median endostylar cilia of *Ciona* usually have straight tips in fresh

Figure 1. DIC flash photographs. A. Living *Spisula* veliger in normal seawater. No paddle cilia are present. Scale bar, 20 μm. B. Living *Lyrodus* veliger in normal seawater. Metachronal waves of pretrochal cilia circle the velum (es, cilia in effective stroke; rs, cilia in recovery stroke). No paddle cilia are evident. Scale bar, 30 μm. C. *Spisula* pretrochal cilia fixed in the hypotonic solution of Campos and Mann (1988) (our Method 1). Cilia have paddle tips. Scale bar, 10 μm. D. *Lyrodus* pretrochal cilia in the hypotonic fixative of Campos and Mann (1988) (Method 1). Vesiculation occurs along the length of the ciliary membranes. Scale bar, 20 μm. E. *Spisula* pretrochal cilia in isosmotic fixative containing 50% seawater (Method 3). No paddle cilia are present. Scale bar, 20 μm. F. *Lyrodus* pretrochal cilia in isosmotic fixative containing 50% seawater (Method 3). No vesiculation of ciliary membranes is evident. Scale bar, 20 μm.

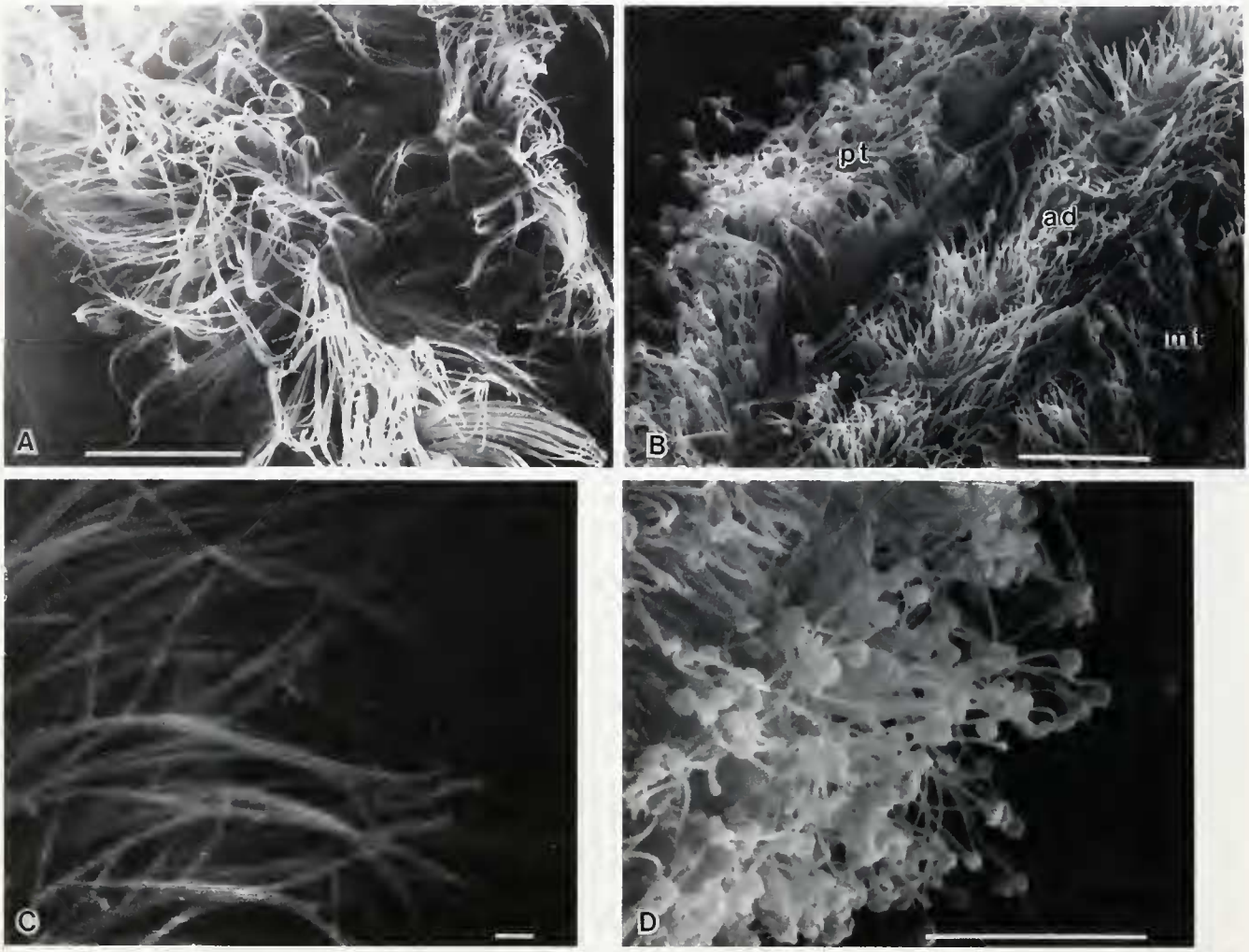

Figure 2. Scanning electron micrographs of velar cilia in *Spisula solidissima* larvae. A, C. Isosmotic fixative containing 50% seawater (Method 3). No paddle cilia are present. B, D. Hypotonic fixative of Campos and Mann (1988) (Method 1). Paddle cilia occur in pretrochal (pt) and metatrochal (mt) bands, but not in the adoral cilia (ad). Scale bars: A, 10 μm; B, 10 μm; C, 1 μm; D, 10 μm.

living preparations. However, the addition of buffered or unbuffered glutaraldehyde fixatives induced rapid coiling of the ciliary tips, resulting in many concentric axonemal coils piled around each other within the ciliary membrane. Coiled ciliary tips were not observed in SEM material that had been quenched in liquid nitrogen and freeze-dried.

The few reports of paddle cilia or discocilia in living preparations in seawater (Heimler, 1978; Arnold and Williams-Arnold, 1980; Matera and Davis, 1982) have been attributed to osmotic stress, anoxia, or other non-physiological conditions in the microscopic slide chambers used for observation (Bone *et al.*, 1982; Pfannenstiel, 1982). In fact, Matera and Davis (1982) induced reversible transitions between paddle cilia and cylindrical cilia by perfusions of hypotonic and hypertonic solutions. In a comprehensive review of the structure of ciliary bands in more than 15 phyla of invertebrates, Nielsen (1987) reported that paddle cilia and discocilia only occur "in specimens which have not been treated with sufficient care." Nielsen (1987) concluded that "until further evidence in favor of paddle cilia in unstressed animals has been presented, I prefer to regard these structures as artifacts."

In this regard, cell physiologists have long recognized that the ciliary membrane is the weakest part of the cilium, and that osmotic stress or non-physiological conditions readily cause coiling or curving of the axoneme within a distal expansion of the ciliary membrane (Hartmann, 1953; Lewin and Meinhart, 1953; Preer and Preer, 1959; Child, 1961; Pitelka and Child, 1964; Mecklenburg *et al.*, 1974). For example, Mecklenburg *et al.* (1974) found that moderate heat exposure caused club-shaped vesicular protrusions of the distal ends of rabbit tracheal cilia. Child

Figure 3. Scanning electron micrographs of velar cilia in *Lyrodus pedicellatus* larvae. A, C. Isosmotic fixative containing 50% seawater (Method 3). No paddle cilia are present. B, D. Hypotonic fixative of Campos and Mann (1988) (Method 1). Paddle cilia are present. Scale bars: A–C, 10 μm; D, 1 μm.

reported that swelling of isolated sucrose-treated cilia of *Tetrahymena* begins first at the tip, and progressively spreads to the base, indicating a "proximal-distal reduction in the strength of connections between the axoneme and the membrane" (Pitelka and Child, 1964, p. 149). Various types of bridges linking the ciliary or flagellar membrane to the outer doublet microtubules have recently been studied biochemically and by electron microscopy (Dentler, 1981). A new mutant of *Chlamydomonas reinhardtii* with disc-shaped flagellar tips (loop-1) similar in appearance to paddle cilia has recently been isolated, indicating a possible genetic defect in the binding between the axoneme and flagellar membrane (Nakamura *et al.*, 1990).

It is commonly observed that paddle cilia and discocilia are limited in distribution within a given specimen; *i.e.*, certain ciliary bands or body regions exhibit modified cilia, whereas other types of cilia in the same animal appear normal (Dilly, 1977a, b; Ehlers and Ehlers, 1978; Heimler, 1978; Arnold and Williams-Arnold, 1980; Bone *et al.*, 1982; Matera and Davis, 1982; Pfannenstiel, 1982; Campos and Mann, 1988; our results). This restricted distribution of paddle cilia and discocilia has been used to argue against their artifactual nature, on the grounds that artifactual production should effect all cilia uniformly (Bergquist *et al.*, 1977; Matera and Davis, 1982; Campos and Mann, 1988). However, Matera and Davis (1982) admitted that, "at the very least, these findings imply some unique properties of the tips of paddle cilia, although they do not alone disprove that the dilations are artifacts."

In fact, workers in the field have long recognized that "different cilia—even on the same organism—are not equally sensitive to stress and some cilia are indeed difficult to fix in a normal shape" (Nielsen, 1987). Our results on differences between ciliary types of veligers in response to hypotonic fixatives supports this finding. It is well-docu-

mented that various types of locomotory and sensory cilia differ in their lipid and protein composition, as well as in the kinds of structures linking the axonemal microtubules to the membrane (Dentler, 1981; Bloodgood, 1990). Therefore, absence of paddle cilia or discocilia in certain types of cilia or body regions of an animal does not mean that modified cilia observed elsewhere on the organism are genuine structures.

The mechanism(s) responsible for the formation of paddle cilia and discocilia is not understood. Our results on living and fixed veligers of *Spisula* and *Lyrodus* suggest that osmotic stress, not the buffers or fixatives used for electron microscopy, is the cause of modified cilia. However, Ehlers and Ehlers (1978) claimed that certain buffers and fixation additives play an important role in generating modified cilia in marine turbellarians. Surprisingly, they found that increasing the osmolality of the fixatives *increased* the numbers of paddle cilia formed.

Convincing evidence that osmotic changes themselves are not required for formation of paddle cilia is Pfannenstiel's (1982) finding that isotonic $MgCl_2$ solution induced paddle cilia in polychaetes. Nevertheless, he also found that the number and time of appearance of modified cilia were inversely related to the concentration of $MgCl_2$.

Bone *et al.* (1982) also discounted osmotic effects as the cause of coiling of ciliary tips in *Ciona*, because the total osmolarity of their glutaraldehyde fixatives was greater than that of seawater, and therefore should have induced a transient shrinkage preceding fixation.

We propose a unifying mechanism for the production of paddle cilia that accounts for many of these seemingly contradictory findings (Fig. 4). We suggest that the primary cause of paddle cilia and discocilia is a conformational change of ciliary doublet microtubules that results in the coiling of the axonemal tip within the distal membrane. Indeed, previous studies indicated that doublet microtubules have an intrinsic tendency to coil when not constrained within the axoneme (Summers and Gibbons, 1971; Zobel, 1973), and that physiological changes in Ca concentration or pH can induce reversible changes in the coiling parameters of isolated doublet microtubules in solution (Miki-Noumura and Kamiya, 1976, 1979; Takasaki and Miki-Noumura, 1982). We recently showed that increased concentrations of Ca, Ba, or Sr induce sharp curvatures of the distal end of axonemes in detergent-extracted macrocilia of *Beroë* (Tamm and Tamm, 1990). This tip curling response is independent of ATP-powered microtubule sliding, and is believed to be caused by Ca/Ba/Sr-induced helical changes in doublet microtubules, some of which are prevented from sliding (Tamm and Tamm, 1990).

In this regard, many of the conditions that induce paddle cilia and discocilia may initially increase Ca or proton flux across the distal ciliary membrane. For example, hypotonic swelling of the tip of the ciliary membrane, where membrane-microtubule bridges are the weakest (Child, 1961; Pitelka and Child, 1964), should increase membrane tension and open stretch-activated ion channels, if present (Guharay and Sachs, 1984; Sachs, 1988). Because stretch-activated channels are cation-selective, and some are Ca-permeable (Christensen, 1987; Lansman *et al.*, 1987), a resulting influx of Ca or change in pH at the ciliary tip might induce a conformational alteration of doublet microtubules that results in coiling of the axonemes (Fig. 4A). Secondly, certain fixatives or chemicals may cause an initial breakdown or permeabilization of the ciliary membrane, leading to similar Ca influx or pH changes which also might trigger conformational changes of the axonemal tip (Fig. 4B). This pathway, it should be noted, would not require distal swelling of the ciliary membrane, and would account for cases of paddle cilia formation under isosmotic or hyperosmotic conditions. Alternatively, disruption of the intact axonemal structure by proteolysis during fixation or handling may remove cross-linking constraints (nexin links, radial spokes) and allow spontaneous conformational alterations of the doublet microtubules, resulting in the coiling of the axoneme (Zobel, 1973) (Fig. 4C). These three possible pathways need not be mutually exclusive; for example, destruction of restraining elements within the axoneme may facilitate Ca or proton-induced alterations in microtubule conformation.

Indeed, Bone *et al.* (1982) found that Ca-blocking agents, such as Co and Mn, reversibly *uncoiled* discocilia in *Ciona*. These authors concluded that discocilia are caused by coiling of axonemes within the ciliary membrane, but believed that such conformational changes were brought about primarily by asymmetrical contraction of the axoneme after cross-linking by glutaraldehyde.

Regardless of the precise pathway(s) involved, the novel feature of the proposed mechanism is an induced or intrinsic conformational change of the doublet microtubules that leads to coiling of the tip of the axoneme (Fig. 4). Dilation or expansion of the ciliary membrane around the looped end of the axoneme would then be merely a passive secondary effect, and not the cause of coiling. Osmotic swelling of the ciliary membrane is thus one method for triggering an ion flux that would induce a conformational change of the doublet microtubules, but membrane tension itself would not be responsible for the coiling of the axoneme.

Our theory for the production of paddle cilia is readily testable. For example, the swollen membrane at the ciliary tip could be disrupted or removed. If the end of the axoneme still remained coiled, then membrane tension is not responsible for maintenance of the paddle. If, on the other hand, the distal end of the axoneme uncoiled and straightened upon disruption of the enclosing membrane, then membrane tension, not intrinsic shape changes of axonemal microtubules, is likely

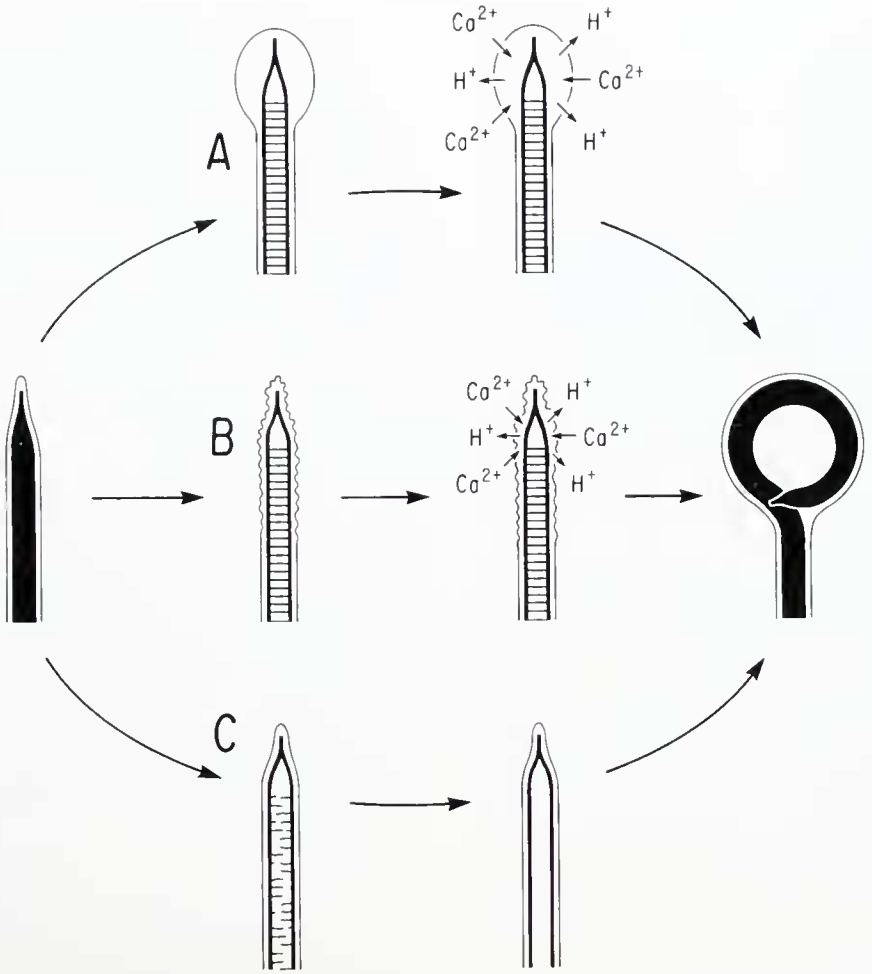

Figure 4. Proposed mechanism of formation of paddle cilia and discocilia. Three possible pathways lead from normal cylindrical ciliary structure (left, straight black axoneme within ciliary membrane) to an induced (A, B) or spontaneous (C) coiling of the axoneme within a distal expansion of the ciliary membrane (right). A. Osmotic swelling by hypotonic solutions or fixatives stretches the distal membrane and opens stretch-activated cation channels; Ca influx or proton efflux trigger conformational changes of the axoneme. B. Fixation or stress initially weakens the ciliary membrane and allows Ca influx or proton efflux, resulting in coiling of the axoneme as in A. No osmotic swelling is necessary. C. Abnormal conditions or fixation lead to weakening or destruction of internal cross-linking restraints (nexin links, radial spokes?), allowing spontaneous conformational alterations of doublet microtubules and coiling of the axoneme. These three pathways need not be mutually exclusive (see text).

to be the cause. Further experiments along these lines are planned.

In conclusion, we believe that our work, together with previous studies, convincingly shows that discocilia and paddle cilia are not genuine structures, but are artifacts. The unifying mechanism we propose to account for their formation suggests that these modifications may be useful for investigating the structural and mechanical properties of axonemal microtubules, as well as the nature of microtubule-membrane interactions in cilia.

Acknowledgments

We thank Scott Gallager, WHOI, for use of facilities, technical assistance, and helpful discussion and advice. We are also grateful to Louie Kerr, MBL EM facility, for SEM assistance, and to Ms. Dorothy Hahn for patiently and skillfully processing these words. This work was supported by a Woods Hole Marine Science Consortium fellowship to G.S. and NIH Grant GM27903 to S.L.T.

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